that Matsumura, et al (1993) teaches α-dystroglycan with molecular weights of 156 kDa and 43 kDa. Matsumura, et al. allegedly further teaches α-dystroglycan from the brain with a molecular weight of 120kDa, and antibodies directed to it. It would therefore be "reasonable to conclude that the antibodies may be used to detect fragments (120 kDa and/or 60 kDa) of α-dystroglycan in blood serum." Paper No. 7, Office Action 4/23/02, page 2, paragraph 4.

Applicants pointed out in their response of 10/2/01 that these proteins are not fragments of α-dystroglycan as recited in Claim 22 (Amended). As pointed out previously, α-dystroglycan from different tissues has different molecular weights. This is in fact noted in the reference. In the present work, epithelial α-dystroglycan with an approximate molecular weight of 180 kDa was exemplified. This leads to fragments of 120 kDa and 60 kDa, as recited in Claims 23 and 24, respectively. These molecular weight fragments are not taught in Matsumura et al. (1993), which does not deal with epithelial cells at all.

Furthermore, Matsumura et al. (1993) do not teach that α-dystroglycan is shed into the serum, as recited in Claim 22 (Amended). The presence of serum indicates a process in a living organism and may be correlated with tumor cell growth, as taught in the specification and recited in Claim 22 (Amended). Matsumura et al. (1993) are concerned with events leading to muscle cell necrosis in the diseases ACARMD and DMD, not tumorigenicity.

Applicants assert that the Examiner is applying an improper "obvious to try" rationale as shown by the reasoning that it would be "reasonable to conclude that that the antibodies may be used to detect fragments (120 kDa and/or 60 kDa) of α-dystroglycan in blood serum." *Id.* The proper rationale is not whether it is "reasonable to conclude" or that it is obvious to combine or try, but instead that there was a suggestion or motivation to combine these references to result in the present invention. MPEP §2145.

There is no motivation in the art to utilize selected antibodies (i.e. IH6) taught by Matsumura et al. (1993) to test for the presence of the α-dystroglycan fragments in blood serum. Assuming that the antibodies disclosed by Matsumura, et al. can be used to detect α-dystroglycan and/or its fragments under the conditions taught by Matsumura, et al., it would not have been

obvious at the time of filing to detect the amount of α-dystroglycan or its fragments found in blood serum because there was no evidence or art that teaches or suggests the existence of dystroglycan in blood serum, or its measure of tumorigenicity. Moreover, it would not have been obvious to measure the size of α-dystroglycan fragments in blood serum because the presence of α-dystroglycan was suggested to be a result of secretion and not as a result of proteolytic cleavage. See Matsumura, et al., at page 13910.

Applicant has amended Claim 22, for the purposes of clarity. Support for this amendment can be found at page 7, first full paragraph beginning at 5, for "an assay of proteolysed α-dystroglycan fragments in blood serum. This assay would add a labeled antibody specific for an α-dystroglycan or a fragment thereof, and assaying for the amount of bound label present in the serum." Further support can also be found at pages 5, 14, 17-19 and 23 in the Specification. Applicants assert that the prior art does not teach or suggest that detecting the presence and size of α-dystroglycan fragments in blood serum indicates tumorigenicity. Therefore, claims 22-24 are not obvious in light of the prior art and Applicants respectfully request the rejection be withdrawn.

2. THE CLAIMS ARE NOT ANTICIPATED

Claims 1-8 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Matsumura, et al. (1997). Specifically the Office Action of 4/23/02 states, that "Matsumura et al. (1997) teach α-dystroglycan, monoclonal antibodies...[and] monoclonal antibodies that specifically bind to it...Matsumura, et al. (1997) further teach the presence of α-dystroglycan and not β-dystroglycan in the culture medium of rat schwannoma cell line RT4, indicating that a fraction of RT4 cell surface α-dystroglycan is dissociated and released into culture medium," Paper No. 7, Office Action 4/23/02, page 3, paragraph 1. The Examiner views the art as anticipatory because it further teaches the presence of dystroglycan in the culture medium, that it can be detected using immunocytochemical analysis and SDS PAGE, and "the role of α-dystroglycan in RT4 cell adhesion to laminin-1, wherein RT4 cells cultured in laminin-1 become

spindle-shaped and adhere to the bottom surface whereas cells inhibited from binding laminin-1 remain rounded." *Id.* The Examiner also asserts that "[i]t is inherent that tumorigenicity potential may be measured by detecting the presence of α-dystroglycan in medium since α-dystroglycan is shed in a tumorigenic cell line (RT4). It is inherent that α-dystroglycan fragments of 60 and 120-130 kDa may be detected since there are monoclonal antibodies directed against α-dystroglycan. It is further inherent that the medium may be blood, tumorigenicity may be measured by detecting the presence or absence of α-dystroglycan on the presence of cells, and by measuring the amount of α-dystroglycan to β-dystroglycan." *Id.*

Applicants respectfully disagree with the characterization of Masumura et al. (1997) as applied to the present claims. It is true that Matsumura et al. (1997) teach monoclonal antibodies to α-dystroglycan and that the secretion of α-dystroglycan by RT4 schwannoma cells into the culture medium. (Schwannoma cells are cells derived from Schwann cells, which form nerve sheaths.)

However, Matsumura at al. (1997) do not teach α-dystroglycan fragments. It therefore follows that the reference does not teach secretion of these fragments into either cell medium (an *in vitro* process) or secretion into blood serum (an *in vivo* process).

Matsumura at al. (1997) do not teach that the presence of α-dystroglycan fragments may be correlated with tumorigenicty. Matsumura et al. demonstrate the rounding up of the Schwannoma cells when blocked with anti- α-dystroglycan antibody. The rounding up occurs because cell adhesion is blocked and the Schwannoma cells cannot flatten out in their normal adherent phenotype. The findings of Matsumura et al. are not associated with tumorigenicity. Contrariwise, Applicants disclose that a tumorigenic cell line lacking α-dystroglycan does round up and form acinar structures, which are associated with normal breast epithelial cells, when transfected with α-dystroglycan. Furthermore, the transfected cells are no longer tumorigenic.

Finally, Matsumura at al. (1997) do not teach detection of a 120 kDa fragment of α-dystroglycan, as recited in Claim 1. They teach the detection of full-size 160 kDa α-dystroglycan (see last paragraph of p. 13905.)

Thus, Matsumura et al. (1997) does not anticipate claims 1-8.

To anticipate an invention, a single prior art reference must disclose each and every limitation of the claimed invention, either expressly or inherently. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." *Continental Can Co v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991). Furthermore, in order for a claim to be inherent in the prior art it is not sufficient that a person following the disclosure sometimes obtain the result set forth in the claim, it must invariably happen. *Glaxo Inc. v. Novopharm Ltd.*, 52 F.3d 1043 (Fed. Cir. 1995).

The teaching of Matsumura, et al. is contrary to the claimed methods because the role of dystroglycan on the cell is taught by the Applicants is to maintain a growth-arrested phenotype (See page 22 and Fig. 3C of the Specification), which includes rounding up and secondary structure (acinii) formation in certain phenotypes (breast tissue). Although Matsumura, et al. (1997) teaches the presence of α-dystroglycan in the culture medium and that it can be detected using immunocytochemical analysis and SDS PAGE, the observation that "the role of α-dystroglycan in RT4 cell adhesion to laminin-1, wherein RT4 cells cultured in laminin-1 become spindle-shaped and adhere to the bottom surface whereas cells inhibited from binding laminin-1 remain rounded" is in direct conflict with what the inventors disclose. Applicants invite the Examiner to compare Figure 4a of Matsumara, et al. (1997) on p. 13907-8, which show the rounded cells of Matsumura, et al. as the phenotype when the IIH6 antibody inhibits the interaction of α-dystroglycan with laminin-1, with Figure 3E-H of the Specification, wherein the inventors show that the rounded phenotype is the normal, non-tumorigenic phenotype that is adopted in response to α-dystroglycan interaction with laminin-1.

Applicants point to the Specification, specifically at Example 5 on page 24-25, wherein the tumor cells lacking α-dystroglycan on the surface, failed to aggregate and round up because they were unable to bind laminin, but remain disorganized and exhibit uncontrolled growth.

Restoration of α-dystroglycan function results in the "rounded" normal phenotype in response to laminin, which is in contrast to the rounded phenotype of the laminin-inhibited, non-adhering cells of Matsumura, et al. (1997). Therefore, Applicants assert that Matsumura, et al. (1997) teaches contrary-wise and away from the invention disclosed and thus, this aspect of the invention is not inherent or anticipated.

Each and every element is not contained in the prior art reference because Matsumura, et al. (1997) also do not disclose that detecting the presence or the size of α-dystroglycan fragments in the cell medium can be used as an indicator of tumorigenic potential. Accordingly, Claims 1-8 are not expressly anticipated by this reference.

Nor is it inherent that "tumorigenicity may be measured by detecting the presence or absence of α-dystroglycan on the surface of cells, and by measuring the amount of α-dystroglycan to β-dystroglycan." There is no suggestion whatsoever concerning the ratio of α-dystroglycan to β-dystroglycan in Matsumura et al. (1997). In addition, tumorigenic potential cannot be invariably measured by detecting the presence or absence of α-dystroglycan on the surface of cells, or by measuring the amount of α-dystroglycan to β-dystroglycan in *all* tumor cell lines by a person practicing the method of Claims 1-8 as shown by the results in Figure 2, lane 2, of BT474 cells. Applicants assert that the Examiner has mischaracterized the invention. Claims 1-8 recite a method for measuring tumorigenic potential, not simply a detection of whether a sample contains cancerous or tumorigenic cells. Applicants' methods correlate the loss of dystroglycan to an observed tumorigenic phenotype and its accompanying properties.

Applicants assert that it is not inherent that all α-dystroglycan fragments may be detected by the monoclonal antibody used by Matsumura, et al. (1997) and directed against α-dystroglycan. Monoclonal antibodies bind their target molecules very specifically as is known in the art. Thus, depending on where the cleavage site of α-dystroglycan is located, the approximately 60 kDa or 120 kDa fragments may not be detectable by an antibody that binds to the fragment of dystroglycan that may remain present on the cell surface and is not present in the

cell medium. Applicants also point to Matsumura, et al. (1997), wherein the effect of different antibodies on cells is shown in Figure 4 (p.13907).

Lastly, because Matsumura, et al. (1997) do not disclose that the presence or the size of α-dystroglycan fragments in the cell medium can be used as an indicator of tumorigenic potential, Claims 1-8 are not anticipated because this element is not contained in the prior art reference. (See Claim 1, step (b)). Moreover, the detection of α-dystroglycan fragments in the cell medium is not inherent to the teachings of Matsumura, et al. (1997). Therefore, Applicants respectfully assert Claims 1-8 are not anticipated and respectfully request that Examiner withdraw this rejection.

No new matter has been introduced by the amendments. The amendments to the specification correct errors in listing products that have been protected by trademark, and to delete duplicated text. One minor error was to correct a mislabeled reference to a figure. Support for the claim amendments can be found at pages 4, 5, 14, 17-19 and 23 of the Specification.

CONCLUSION

Applicants urge the Examiner to withdraw all rejections. Applicants hereby request a One-Month Extension of time from July 23, 2002 to August 23, 2002. A petition for a one month extension of time is included herewith in duplicate. Please charge any necessary and additional fees that may be due to Deposit Account No. 12-0690.

For the reasons set forth above, Applicants respectfully request that a timely Notice of Allowance be issued in this case. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned at (510) 495-2839.

Respectfully submitted,

Dated 8-15-02 BY _____

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Appendix 1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Please replace paragraph 2 on page 18 which continues onto page 19 with the following rewritten paragraph:

After separation on an 8% SDS-PAGE gel, the proteins were electrophoretically transferred to ~~Immobilon-P~~ IMMOBILON-P membranes (Millipore Corp., Bedford, MA) using methanol transfer buffer (20 mM Tris pH 8.3, 150 mM glycine, 0.5% SDS, 20% methanol). Following the transfer, the membranes were blocked for two hours in low salt TBST buffer (20 mM Tris-HCL, pH 8.0, 100 mM NaCl, 1.0% Tween-20 detergent) plus 5% non-fat dried milk (Lucerne). After blocking the membranes were incubated for 45 minutes with the IIH6 monoclonal antibody (primary antibody against α-dystroglycan, obtained from Dr. Kevin Campbell, University of Iowa) diluted in low salt TBST plus 5% dried milk. The primary antibody was diluted 1:20 from a concentrated (8X) stock of hybridoma supernatent. After incubation with the primary antibody, the membranes were washed with low-salt TBST and incubated 45 minutes with an horse radish peroxidase (HRP) conjugated secondary antibody (anti-IgM Product # A 8786 from Sigma). The membranes were thoroughly washed for 1 hour after the secondary antibody and the signal detected by chemiluminescence ~~Supersignal~~ SUPERSIGNAL substrate (Pierce, Rockford, IL) and exposure to film. α-dystroglycan that is cleaved and shed from the cell surface appears as a distinct 130 kilodalton (kD) band in the medium (Figure 1A), whereas ~~β-dystroglycan~~ α-dystroglycan isolated from the cell surface migrates as a broad 180 kD band (Figure 1A "Cell").

Please delete paragraph 2, which is Example 4, beginning at page 23, as it is in duplicate of Example 4 on page 24 of the specification.

-- ~~Example 4. In vitro assays of α-dystroglycan proteolysis.~~

~~The α-dystroglycan protein, or derivative thereof, is used as a substrate for a cell-free assay measuring the activity of the protease(s) cleaving it. The substrate consists of either the full-length α-dystroglycan molecule, a fragment thereof, or a synthetic peptide capable of being recognized and cleaved by the enzyme cleaving α-dystroglycan. Detection of the cleavage event is assayed by any of several methods existing in the art. These methods include, but are not limited to: immunoblotting with α-dystroglycan-specific antibodies to detect proteolytic fragments, detection of radiolabeled fragments, detection of flurogenic peptide cleavage. These assays can easily be modified and optimized by a variety of methods existing in the art.~~ --

In the claims:

Please amend Claim 22 with the following changes:

22. A method of assaying proteolysed α-dystroglycan fragments shed from a cell into blood serum comprising the steps of:

a. contacting a serum sample to be assayed with a labeled antibody specific for an α-dystroglycan fragment, and

b. assaying the amount of bound label~~,~~

wherein said α-dystroglycan fragments bound to said labeled antibody are positively correlated with tumor cell growth.

Please add the following claims:

29. The method of claim 22, wherein said cell is an epithelial cell.

30. The method of claim 29, wherein said epithelial cell is a breast epithelial cell.

Appendix 2

1. A method for measuring potential tumorigenicity of mammalian cells comprising:

   a. providing a tissue sample or sample of medium surrounding cells, and

   b. detecting the presence of a fragment of α-dystroglycan in medium, said fragment having an Mr of 120-130kD, whereby the presence of the fragment indicates higher potential tumorigenicity.

2. The method of claim 1, wherein said detecting comprises:

   a. adding to said sample a material selected from the group consisting of a monoclonal antibody to α-dystroglycan and laminin, and

   b. measuring the size of the α-dystroglycan fragment detected.

3. The method of claim 1, wherein said cells are human mammary epithelial cells.

4. The method of claim 1, wherein said medium is blood serum.

5. A method for measuring potential tumorigenicity of cells, comprising:

   a. providing a sample of said cells, and

   b. detecting the presence of α-dystroglycan on the surface of the cells, whereby the absence of α-dystroglycan indicates a higher potential for tumorigenicity.

6. The method of claim 5, wherein said detecting comprises:

   a. adding to said sample a monoclonal antibody to α-dystroglycan, and

   b. measuring the amount of labeled α-dystroglycan detected.

7. The method of claim 5, wherein said cells are human mammary epithelial cells.

8. The method of claim 5, wherein said detecting comprises measurement of the amount of α-dystroglycan relative to the amount of β-dystroglycan, wherein a relative decrease of α-dystroglycan indicates α-dystroglycan shedding and higher potential tumorigenicity.

22. A method of assaying proteolysed α-dystroglycan fragments shed from a cell into blood serum comprising the steps of:

a. contacting a serum sample to be assayed with a labeled antibody specific for an α-dystroglycan fragment, and

b. assaying the amount of bound label,

wherein said α-dystroglycan fragments bound to said labeled antibody are positively correlated with tumor cell growth.

23. The method of Claim 22, wherein the α-dystroglycan fragment is an approximately 120 kD fragment.

24. The method of Claim 22, wherein the α-dystroglycan fragment is an approximately 60 kD fragment.

29. The method of claim 22, wherein said cell is an epithelial cell.

30. The method of claim 29, wherein said epithelial cell is a breast epithelial cell.